Refer to: Mahley RW: The role of dietary fat and cholesterol in atherosclerosis and lipoprotein metabolism—Medical Staff Conference, University of California, San Francisco. West J Med 134:34-42, Jan 1981

# The Role of Dietary Fat and Cholesterol in Atherosclerosis and Lipoprotein Metabolism

*These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California, San Francisco. Taken from transcriptions, they are prepared by Drs. David W. Martin, Jr, Professor of Medicine, and James L. Naughton, Assistant Professor of Medicine, under the direction of Dr. Lloyd H. Smith, Jr, Professor of Medicine and Chairman of the Department of Medicine. Requests for reprints should be sent to the Department of Medicine, University of California, San Francisco, School of Medicine, San Francisco, CA 94143.*

DR. SMITH:* *The topic of this discussion is the role of dietary fat and cholesterol in atherosclerosis and lipoprotein metabolism. This gives us the opportunity to introduce an important new member of our faculty, Dr. Robert W. Mahley. Dr. Mahley received his medical degree and doctorate from Vanderbilt University. Much of his career has been spent at the National Institutes of Health where most recently he has been head of the Comparative Atherosclerosis and Arterial Metabolism Section in the National Heart, Lung, and Blood Institute. Last fall, Dr. Mahley came to UCSF as Professor of Pathology, with collateral appointment in medicine. Here, he heads an important new institute, the Gladstone Foundation Laboratories for Cardiovascular Disease located at San Francisco General Hospital.*

DR. MAHLEY: In the summer of 1980 the newspapers and popular magazines gave extraordinary coverage to a new national report from the Food and Nutrition Board of the National Academy of Sciences. The study concluded that available research data do not support the link between the consumption of dietary cholesterol and coronary artery heart disease and that there is no need for the average healthy person to restrict consumption of high fat, high cholesterol foods.[1]

The controversy has expanded and the waters have been muddied by claims of an apparent conflict of interest in the association between some of the board members and the food industry. A couple of the scientists are associated with, or have been consultants for, the meat, dairy and egg industries, and a couple of board members are executives of commercial food companies. The public has been confused by this controversy and by the charges of a conflict of interest, and both the public media and people in general are questioning the credibility of the medical and scientific community. The discussion and the debate on the role of dietary fat and cholesterol are not the problem—this is the way of science. The confusion, however, has undermined scientific evaluation in the public's view and this is at least partially because the panel apparently did not consider all the available evidence on the subject.

In my opinion, the panel overstated its conclusions and ignored a large and persuasive body

*Lloyd H. Smith, Jr, MD, Professor and Chairman, Department of Medicine.

ABBREVIATIONS USED IN TEXT

CHD = coronary heart disease
HDL = high-density lipoproteins
$HDL_c$ = cholesterol-induced high-density lipoproteins
$HDL_1$ = high-density lipoproteins containing E apoprotein
$HDL_2$ = high-density lipoproteins lacking E apoprotein
LDL = low-density lipoproteins
VLDL = very-low-density lipoproteins

of evidence that shows a clear relationship between plasma cholesterol levels, the intake of dietary fat and cholesterol and accelerated heart disease. When one considers the entire body of data, including epidemiologic and population studies, clinical trials and diet experiments in animals, the evidence overwhelmingly supports the idea that diet and heart disease are strongly linked. I want to review only a few selected data for you.

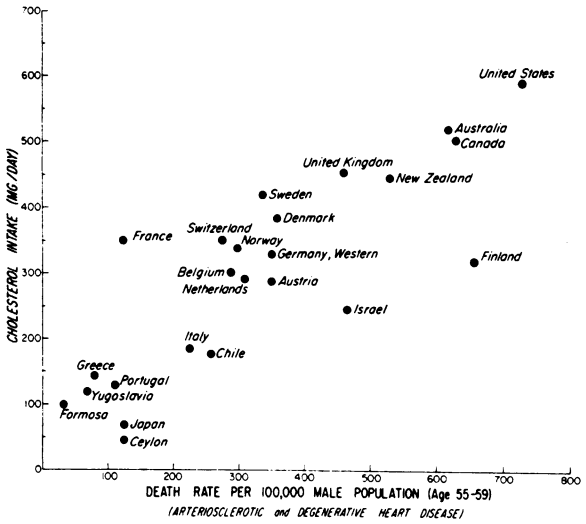


**Figure 1.**—Death rates from coronary heart disease for the mean of men aged 55 to 59 years in 24 countries significantly correlated with the mean daily intake of cholesterol in the diet. (Reproduced with permission from Connor and Connor.[2])

## Epidemiologic and Population Studies

A positive correlation between certain dietary factors and mortality from coronary heart disease (CHD) is evident from various epidemiologic studies. Vital statistics from 30 countries provided by the World Health Organization (WHO) and Food and Agricultural Organization (FAO) of the United Nations indicate that there is a positive correlation between mortality and intake of total calories, total fat, animal fat, meat, cholesterol, eggs and animal protein in men 55 to 59 years of age. There is either no correlation or a negative correlation between coronary heart disease and the intake of vegetable fat, vegetables and fish. As shown in Figure 1, arteriosclerotic and degenerative heart disease are strongly correlated with cholesterol intake. Populations consuming, on the average, more than 500 mg of cholesterol per day (such as in the United States, Australia and Canada) have a death rate per 100,000 male population (aged 55 to 59 years) that is many times greater than that in populations consuming less than 150 mg of cholesterol per day (for example, in Japan).[2]

A positive association between dietary saturated fat and atherosclerotic disease is also clearly shown in other epidemiologic studies. The International Cooperative Study on Epidemiology of Cardiovascular Disease, a prospective study of 40- to 59-year-old men from seven countries, showed that the percentage of total calories provided by saturated fatty acids correlates significantly with the incidence of fatal and nonfatal myocardial infarctions. Serum cholesterol levels correlate with both the consumption of saturated fat and the incidence rates.[3] It should be noted that diets high in saturated fats usually contain high levels of cholesterol as well because both occur in similar foods of animal origin.

Population studies link changes in dietary habits with elevations in plasma cholesterol levels and with the occurrence of coronary artery disease. One such study correlates the incidence of coronary artery disease with increased dietary lipid intake and elevated serum cholesterol levels in Japanese men living in Japan or emigrating to the United States (Hawaii or California). Of all the industrialized countries of the world, Japan has one of the lowest rates of coronary heart disease, as shown in Figure 1. This study, begun in 1965, indicates that as Japanese men are assimilated into the westernized society of the United States, they increase their intake of animal products including total fat, saturated fat and cholesterol (Table 1). Associated with the dietary changes, there is an increase in serum cholesterol levels and an increase in CHD. The incidence of deaths due to myocardial infarction and CHD is lowest for native Japanese men and highest for Japanese men living in California.[4]

Changes in diets associated with war-time populations have also provided evidence to support

links between diet and heart disease. During World War II, the invasion and occupation of various Scandinavian countries produced a sharp decline in the consumption of animal fats, eggs and dairy products, and a decline in the death rate from coronary artery disease. In Norway and Finland where shortages of food came early in the war, the decline in CHD also came early. In the Netherlands, however, where the change in eating patterns occurred late in the war, the decline in CHD occurred much later.[5]

Numerous other epidemiologic studies, such as the detailed reviews of Keys[3] and Stamler,[4] give a more complete discussion. However, it is important to point out that such studies do not prove a direct cause and effect relationship between CHD and dietary fats. Like all scientific data, epidemiologic studies must be considered in the light of all available evidence. They do, however, strongly support the diet-lipid-heart disease hypothesis. Atherosclerosis is a complicated disease process upon which we must bring the tools of all disciplines to bear if we are to understand its pathogenesis. Epidemiologic data represent pieces of a very complex puzzle.

Another piece of the puzzle comes from diet studies in animals. These studies appear to have been ignored by the Food and Nutrition Board in their deliberations. Diets high in fat and cholesterol will lead to accelerated atherosclerosis in essentially every animal species that has been studied, and I suggest that humans are no exception.

## Animal Studies

One can create dietary and metabolic conditions which will induce, in most animals under study, a form of atherosclerosis that resembles certain aspects of the disease process as it occurs in humans.[6] However, such animal experiments provide us with a slightly different view of the disease than as it occurs in humans. Figure 2 shows a histologically prepared cross section of the anterior descending coronary artery from a cholesterol-fed miniature swine. This animal had had a hypercholesterolemia of 400 mg per dl for about a year. The lumen of the vessel shown in Figure 2 is significantly (60 percent to 70 percent) narrowed by a very typical atheromatous lesion. A characteristic of atherosclerosis in all animal species is the deposition of cholesterol in and around cells of the arterial wall. The cholesterol that accumulates is derived from plasma lipoproteins, and it is important to identify the specific lipoproteins responsible for the delivery of cholesterol to cells and to identify the cell type that accumulates the cholesterol.

It is our contention that animal studies are providing important data that are relevant to humans. They are relevant because they show that high levels of dietary cholesterol change the cholesterol-carrying plasma lipoproteins and that this causes the lipoproteins to deliver cholesterol to the arterial wall. *How the cholesterol is transported and by which lipoproteins may be more important than the absolute level of the plasma cholesterol.* To conclude that a particular dietary regimen (high or low in a specific constituent) is unimportant because it has little or no effect on the total plasma cholesterol, reflects a lack of appreciation of the dynamics of lipoprotein-cholesterol metabolism. The various plasma lipoproteins of humans and animals all transport cholesterol and represent a metabolically diverse group of macromolecules that may have entirely opposite effects on the ingress or egress of cholesterol in the

TABLE 1.—*Dietary Changes Associated With the Emigration of Japanese Men to the United States**

| | *Japan* | *Hawaii* | *California* |
|---|---|---|---|
| Diet | | | |
| Total calories | 2,164 ±619 | 2,275 ±736 | 2,262 ±695 |
| Animal protein (g) | 39.8± 22.8 | 70.5± 32.7 | 66.0± 24.4 |
| Total fat (g) | 36.6± 20.4 | 85.1± 38.9 | 94.8± 36.4 |
| Saturated fat (g) | 16.0± 13.3 | 59.1± 32.7 | 66.3± 30.5 |
| Cholesterol (mg) | 464.1±324.4 | 545.1±316.4 | 533.2±297.8 |
| Serum cholesterol | | | |
| mg/dl | 181.1± 38.5 | 218.3± 38.2 | 228.2± 42.2 |
| >260 mg/dl (per 1,000) | 31.6 | 124.0 | 162.5 |
| Myocardial infarction and coronary heart disease deaths | | | |
| Incidence compared with Japanese men in Japan | .... | Twofold | Threefold |

*Modified from Stamler.[4]

arterial wall. An understanding of the diversity of roles of specific lipoprotein classes and subclasses has only begun.

A provocative study by Armstrong and coworkers[7] in monkeys offers an excellent introduction to the value of using animals to study the role of diet in the pathogenesis of atherosclerosis. In this study, rhesus monkeys were fed cholesterol-free or cholesterol-supplemented diets for 18 months. Monkeys fed small amounts of cholesterol had plasma cholesterol levels that were matched with monkeys on the cholesterol-free diet (all monkeys had cholesterol levels of less than 220 mg per dl). At the end of the study, monkeys that received cholesterol in their diets had much more severe atherosclerosis than did the monkeys on the cholesterol-free diet, despite the fact that both groups had the identical plasma cholesterol levels. Adding cholesterol to the diet increased the deposition of cholesterol in the arterial wall without producing an excessive hypercholesterolemia.

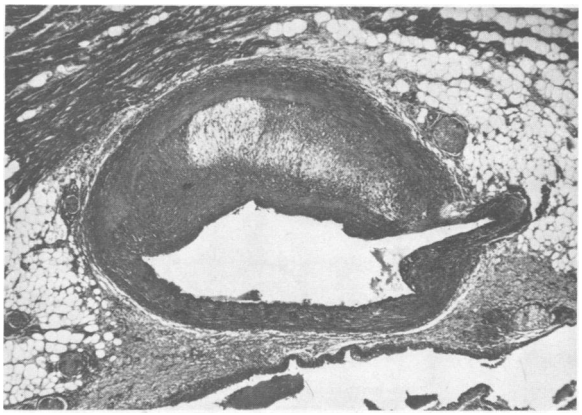

**Figure 2.**—A cross section of the anterior descending coronary artery from a miniature swine fed a high fat, high cholesterol diet (15 percent lard, 1 percent cholesterol by weight added to a standard chow) for one year. The atherosclerotic lesion has partially occluded the lumen of this major coronary artery.

How did more severe atherosclerois develop in the monkeys consuming the cholesterol-supplemented diet when their plasma cholesterol levels were the same as those of the control (noncholesterol-fed) monkeys? What is the effect of the dietary cholesterol? It is reasonable to speculate that dietary cholesterol (exogenous) is metabolized differently from cholesterol synthesized in the body (endogenous). It is our contention that the intake of dietary cholesterol causes changes in the types and metabolism of plasma lipoproteins, perhaps very subtle changes, or changes that we have not yet identified, but changes, nevertheless, that lead to deposition of cholesterol in tissues.

## Changes in Lipoproteins

I wish to describe some of the changes that occur in the lipoproteins after cholesterol feeding and that are associated with accelerated atherosclerosis in nonhuman species of animals. Data

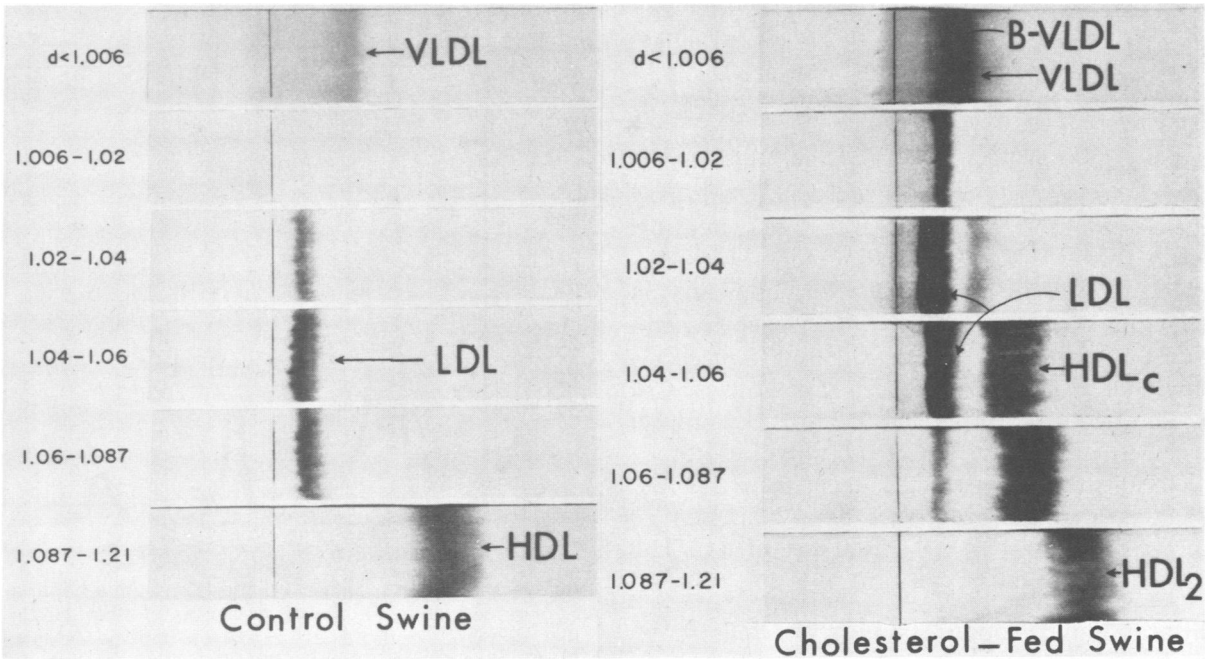


**Figure 3.**—Comparison of the plasma lipoproteins from a control and a cholesterol-fed miniature swine. (Reproduced with permission from Mahley et al.[9])

show that similar changes in the lipoproteins occur in humans on a high cholesterol diet. Regardless of the species of animal, there are certain consistent changes in the lipoproteins associated with accelerated atherosclerosis induced by the high fat, high cholesterol diets.[3,8]

Figure 3 illustrates the changes that occur by comparing the lipoproteins from a control miniature swine with those from a cholesterol-fed swine. The control swine has lipoproteins very similar to the lipoproteins that occur in humans, including very-low-density lipoproteins (VLDL, or pre-$\beta$-lipoproteins), low-density lipoproteins (LDL, or $\beta$-lipoproteins) and high-density lipoproteins (HDL, or $\alpha$-lipoproteins). The atherogenic hyperlipidemia that occurs with cholesterol feeding is characterized by four changes. First, there is the appearance of $\beta$-VLDL (or B-VLDL) in the $d<1.006$ ultracentrifugal fraction. The B-VLDL are cholesterol-rich lipoproteins similar to the B-VLDL that occur in patients with type III hyperlipoproteinemia or dysbetalipoproteinemia. Normally, the $d<1.006$ fraction contains only the pre-$\beta$-migrating, triglyceride-rich VLDL. The B-VLDL are $\beta$-migrating, cholesterol-rich lipoproteins. We have seen this change in every species studied. The second change that occurs with cholesterol feeding is an increase in the LDL, and the third is the appearance of $\alpha_2$-migrating, cholesterol-rich lipoproteins, referred to as $HDL_c$. Our data indicate that $HDL_c$ are derived from the typical plasma HDL as they pick up cholesterol from peripheral cells. As the typical HDL become loaded with cholesterol, they increase in size and float at lower densities, becoming the $HDL_c$. The subscript *c* indicates that the lipoproteins are cholesterol-induced. We believe that the $HDL_c$ transport the cholesterol from peripheral tissues, such as the arterial wall, to the liver for excretion. The presence of a specific protein (the E apoprotein) on the $HDL_c$ serves as the signal for hepatic recognition and uptake.[10,11] By typical HDL, I mean $\alpha_1$-migrating, small, protein-rich lipoproteins that usually float at a density of 1.063 to 1.21. A fourth change that occurs is a decrease in typical HDL.

In all the species studied, then, there are four changes: (1) the occurrence of B-VLDL, (2) an increase in LDL, (3) the appearance of $HDL_c$ and (4) a decrease in typical HDL.

## Interaction of Lipoproteins With Cells

How do these various lipoproteins interact with cells to regulate cholesterol metabolism? The scheme by which various cells metabolize cholesterol is shown in Figure 4. Fibroblasts and smooth muscle cells have on their surfaces specific high-affinity cell surface receptors that recognize the LDL.[12] This recognition of the lipoproteins by the cell receptors starts a series of intracellular events in which the LDL cholesterol is internalized and hydrolyzed, and by which cholesterol metabolism is regulated. Not only do low-density lipoproteins (which contain the B apoprotein) interact with these cell surface receptors, but certain high-den-

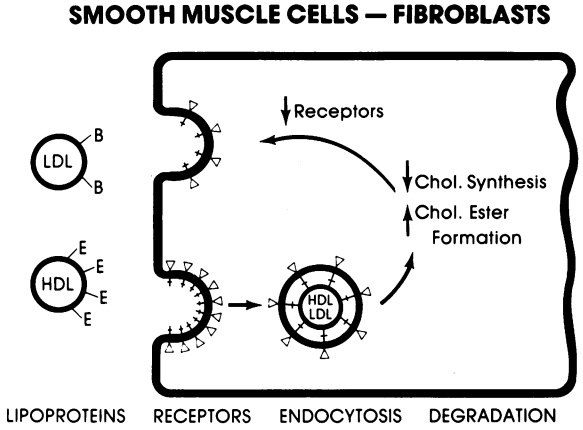


**Figure 4.**—Schematic diagram illustrating the cell surface receptor binding of the B apoprotein-containing LDL and the E apoprotein-containing HDL. Typical HDL lacking the E apoprotein do not bind. Intracellular cholesterol metabolism is regulated by these lipoproteins. (Chol = cholesterol; HDL = high-density lipoproteins; LDL = low-density lipoproteins.)

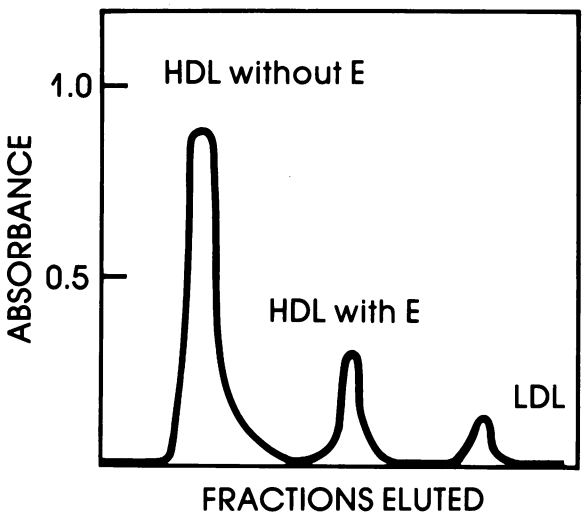


**Figure 5.**—Elution profile of human HDL from a heparin-Sepharose affinity column. (E = the E apoprotein; HDL = high-density lipoproteins; LDL = low-density lipoproteins.) (See Weisgraber and Mahley[15] for complete description of methodology and for characterization of the subclasses.)

sity lipoproteins (those containing a certain type of protein called the E apoprotein) also interact with the same receptor sites and lead to intracellular regulation of cholesterol metabolism. By contrast, the typical HDL do not interact with cell receptors.[10,13,14]

I must stress that our studies show two distinct types, or subclasses, of HDL—each with different metabolic properties (Figure 5). One subclass of HDL is capable of binding to the cell surface receptors on fibroblasts and smooth muscle cells, the same receptor site that interacts with LDL. This subclass is the HDL with the E apoprotein; however, the second subclass of HDL lacks the E apoprotein (Figure 5) and does not bind to the cell surface receptors of fibroblasts and smooth muscle cells. These two subclasses of HDL occur in both humans and experimental animals and can be quantitatively isolated using heparin-Sepharose affinity chromatography.[15,16]

It is the HDL with the E apoprotein that increase with cholesterol feeding and become the $HDL_c$. This has been shown in several species of animals in our laboratory and more recently has become evident in studies of humans who have consumed high cholesterol diets. The changes in the HDL following cholesterol feeding are illustrated in Figure 6. In this study we compared the two HDL fractions from a dog fed a low cholesterol (control) diet and then a high cholesterol diet. After cholesterol feeding, the second HDL peak (the HDL with the E apoprotein) was increased. In this particular example, the HDL with E apoprotein (the $HDL_c$) increased fourfold within four days after cholesterol feeding began. Not only did the mass of HDL with E increase, but the concentration of the E apoprotein in this particular lipoprotein also increased.[16]

In studies of normal humans, we have shown that binding activity of HDL to the cell surface receptors of fibroblasts can be increased three- or fourfold by eating a high cholesterol diet in the form of four to six eggs per day.[17] In Figure 7 the binding activity of the HDL before and after cholesterol feeding are compared in two subjects. The binding activity of the HDL from both subjects increased after the addition of the cholesterol to the diet. This is an indication of the more direct data that we now have showing that the $HDL_c$ (the HDL with the E apoprotein) are increasing in human plasma just as we have shown in the several animal species (E. Flaim, T. P. Bersot and R. W. Mahley, in preparation). We have now studied over a dozen such subjects with similar results.

It is important to note that the changes in the

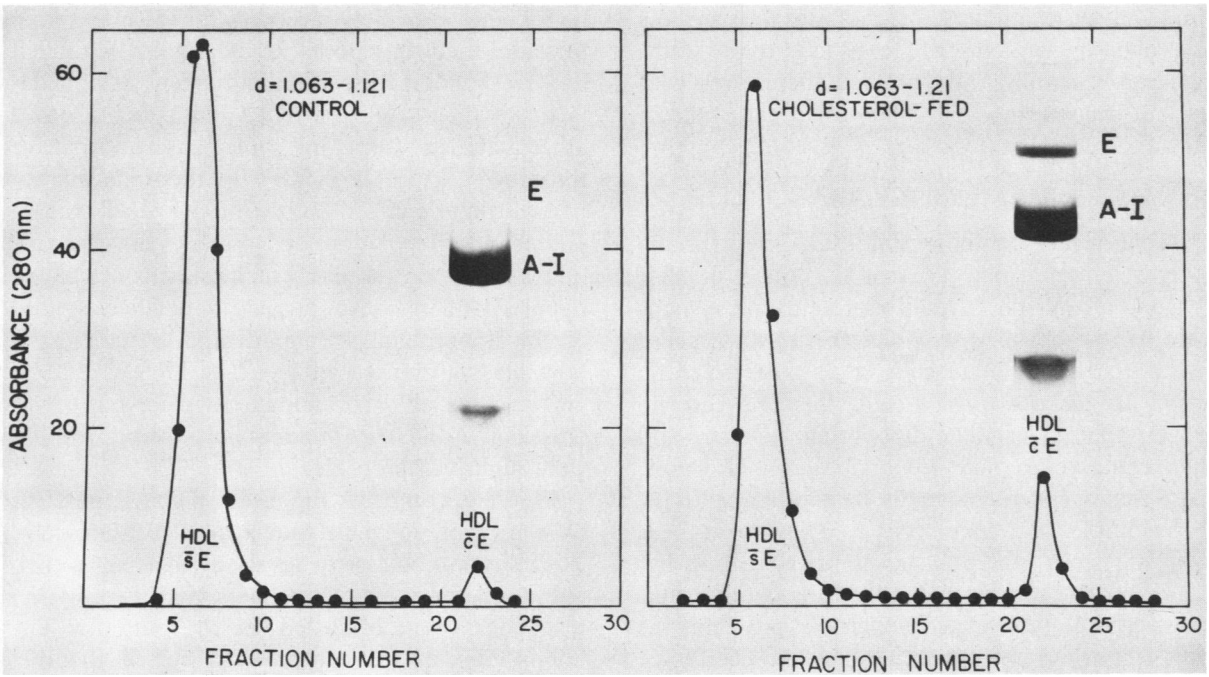


**Figure 6.**—Elution profile from a heparin-Sepharose affinity column of canine HDL (d = 1.063-1.21) before (control) and after four days of cholesterol feeding. ($HDL_{\bar{c}}E$ = high-density lipoproteins with E apoprotein; $HDL_{\bar{s}}E$ = high-density lipoproteins without E apoprotein.)

HDL occurred without regard to plasma cholesterol levels (Figure 7). In both subjects the plasma cholesterol was approximately 200 mg per dl before the high cholesterol diet. During the four-week feeding study, the plasma cholesterol in subject 1 did not change at all. In subject 2, the plasma cholesterol rose by 23 percent. However, in both subjects a change in the metabolic activity of the HDL occurred. Therefore, subtle metabolic changes can occur in specific lipoproteins with or without gross changes in the cholesterol content of the plasma, and these changes can affect the ways in which these lipoproteins interact with cells and regulate cholesterol metabolism.

Thus, by studying the various normal and cholesterol-induced lipoproteins, we have found that LDL and certain types of HDL (HDL containing the E apoprotein) can bind to receptors on the surface of the cells and regulate intracellular cholesterol metabolism in fibroblasts and arterial smooth muscle cells. These studies show much about how lipoproteins interact with cells. However, it is hard to explain atherosclerosis on the basis of these results. The receptor-mediated uptake process is self-regulating; a feedback system allows fibroblasts and smooth muscle cells to take up only a limited amount of cholesterol before the availability of cell surface receptors is repressed. Smooth muscle cells and fibroblasts will not accumulate large amounts of cholesterol regardless of which lipoproteins are exposed to the cells. Thus, they simply will not load themselves with fat and become the lipid-laden foam cells seen in the atherosclerotic lesion.

## The Macrophage and B-VLDL

Then, what are those lipid-filled cells in the atherosclerotic lesion? What is the source of their cholesterol? Which of the lipoproteins are responsible for the delivery of cholesterol to these cells? If the lipid-laden foam cells in an atherosclerotic lesion are smooth muscle cells, then they are no longer able to regulate lipoprotein uptake, and thus continue to take up cholesterol. However, these foam cells may actually be another cell type, for example, *the macrophage*. That foam cells originate from macrophages is an appealing hypothesis. The cholesterol-fed dog and other animals used in our experiments are providing us with important information about the cell type and the lipoproteins involved. The cells that accumulate the cholesterol strongly resemble macrophages, as do the cells that occur in certain types

**CHOLESTEROL INDUCED CHANGES IN HUMAN HDL**

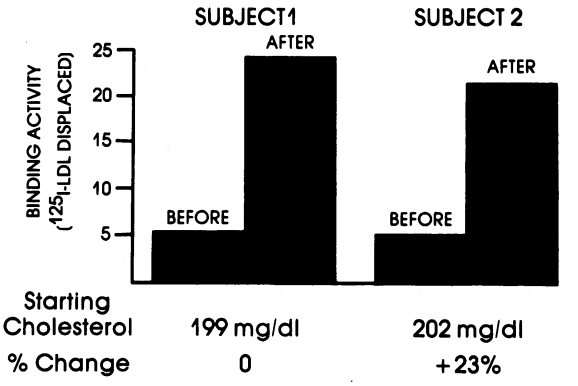


**Figure 7.**—Ability of human high-density lipoproteins (HDL, d = 1.095-1.21) to compete with low-density lipoproteins (LDL) labeled with iodine 125 for binding to the cell surface receptors of cultured human fibroblasts. Comparisons made between HDL obtained before and after addition of 4 to 6 eggs per day to both subjects' diets for four weeks. (See Mahley et al[17] for description of results.)

of human atherosclerosis. The lipoproteins that deposit cholesterol and accelerate atherosclerosis appear to be the $\beta$-very-low-density lipoproteins (B-VLDL).

Dogs do not naturally develop any arterial lesions that can be called atherosclerosis. Atherosclerosis can be induced in dogs only when the plasma cholesterol level is maintained by high fat, high cholesterol diets in excess of 750 mg per dl for more than six months. In these animals (called hyperresponders), severe lipid-laden lesions develop, which are associated with complicated atherosclerosis and, in some cases, occlusive thrombosis.[13,18,19] In dogs with diet-induced hypercholesterolemia of 350 to 700 mg per dl atherosclerosis does not develop at all, even after one or two years on the diet (such animals are called hyporesponders). We turned to detailed studies of the lipoproteins in an attempt to understand how plasma cholesterol levels as high as 350 to 700 mg per dl were nonatherogenic, but levels only slightly higher (in excess of 750 mg per dl) were highly atherogenic.

Figure 8 compares the electrophoretic patterns of a control dog and four cholesterol-fed dogs. The plasma cholesterol levels of the cholesterol-fed dogs (two hyporesponders and two hyperresponders) are indicated on the patterns. The lipoproteins of the control dog include the LDL and two $\alpha$-migrating lipoproteins referred to as $HDL_1$ and $HDL_2$. $HDL_1$ are the high-density lipoproteins that contain the E apoprotein, and the $HDL_2$

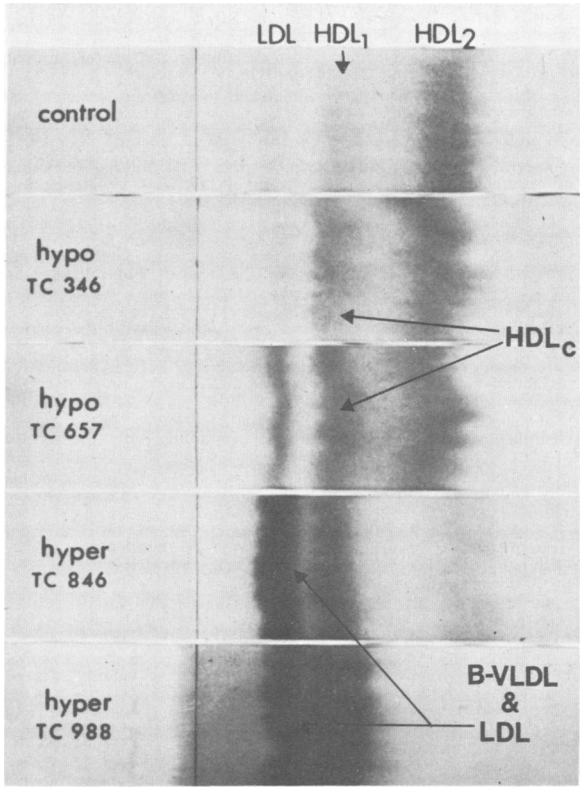


**Figure 8.**—Paper electrophoretograms of the plasma lipoproteins of a control dog (top strip) and four different cholesterol-fed dogs with variable degrees of hypercholesterolemia (HDL = high-density lipoproteins; LDL = low-density lipoproteins; TC = total plasma cholesterol in mg/dl). (Reproduced with permission from the American Heart Association, Inc.; from Mahley et al.[20])

are the high-density lipoproteins that lack the E apoprotein. As plasma cholesterol levels increase with cholesterol feeding, the principal lipoprotein to carry the cholesterol is the one with $\alpha_2$-mobility like the HDL$_1$. In the cholesterol-fed dog, HDL$_1$ is the HDL$_c$. Also, as the plasma cholesterol levels increase, the LDL concentration increases. Thus, in the hyporesponders, there are two major changes: an increase in the LDL and the appearance of the HDL$_c$ and, remember, no atherosclerosis.[13]

The dogs in which atherosclerosis developed (the hyperresponders with plasma cholesterol levels in excess of 750 mg per dl) present a more complicated electrophoretic pattern. There is a broad $\beta$, pre-$\beta$ band. This correlates with the presence of the B-VLDL, already discussed. These animals also have an increase in the LDL. As can be noted in Figure 8, the HDL$_c$ remain prominent, and there is a reduction in the typical HDL. At the risk of oversimplification—there are two major differences between the nonatherogenic hyperlipidemia and the atherogenic hyperlipidemia. These are the appearance of B-VLDL and the decrease in the typical HDL.

Our attention has focused in recent months on the B-VLDL as a likely candidate for the atherogenic lipoprotein and on *the macrophages* as the cells likely to accumulate the cholesterol in the arterial wall. We designed studies to determine if any of the normal or cholesterol-induced lipoproteins are taken up by macrophages in culture. We found that, of all the naturally occurring lipoproteins, only the B-VLDL will cause cholesterol to accumulate in macrophages. VLDL, LDL and HDL from normal animals and LDL and HDL$_c$ from cholesterol-fed animals will not cause the massive deposition of cholesterol in macrophages. Cholesterol deposition (primarily cholesteryl esters) occurs only from the B-VLDL. Macrophages incubated with B-VLDL have a 20- to 160-fold increase in cholesteryl ester content. The uptake is mediated by a high-affinity cell surface receptor that is specific for B-VLDL. In cholesterol-fed dogs, the B-VLDL cause the accumulation of cholesterol; these lipoproteins are also responsible for such accumulation in cholesterol-fed monkeys, rats and rabbits. The B-VLDL from all of these species load macrophages with cholesteryl esters.[13]

Preliminary data suggest that cholesterol feeding in humans causes the appearance of lipoproteins resembling B-VLDL. These lipoproteins may be present transiently in the plasma after a high fat, high cholesterol meal. It is reasonable to speculate, on the basis of the animal studies, that these lipoproteins (B-VLDL) may appear repeatedly as we overindulge in high fat, high cholesterol meals, and that over the years this could lead to deposition of cholesterol in the arterial wall. These studies in animals and humans suggest that high levels of dietary lipids alter several different types of plasma lipoproteins, change the way cholesterol is transported in the plasma and alter the metabolic manner in which these various lipoproteins interact with cells. High levels of dietary cholesterol cause some of these changes in plasma lipoproteins with or without altering the plasma cholesterol levels.

## Summary

When diets high in fat and cholesterol are fed to different species of animals, they cause significant changes in various classes of lipoproteins, including the appearance of B-VLDL, an increase in LDL, the appearance of HDL$_c$ and the decrease in typical HDL. Similar, but more subtle changes,

are being observed in humans—such as the role of these lipoproteins in the cellular metabolism of cholesterol. Smooth muscle cells and fibroblasts take up B-VLDL, LDL and $HDL_c$ via specific cell surface receptors that recognize the lipoproteins that contain the B and E apoproteins. However, the amount of cholesterol that can accumulate in smooth muscle cells or fibroblasts is closely regulated, preventing the conversion of these cells to the lipid-laden foam cells characteristic of atherosclerosis. It is intriguing that macrophages, however, do accumulate large amounts of cholesteryl esters when presented with the cholesterol-induced B-VLDL from various species, but not in response to any of the other naturally occurring lipoproteins.

I believe that when one looks at all of the pieces of the puzzle, including population and epidemiologic data and diet experiments in animals, it is evident that dietary fat and cholesterol are, indeed, linked to accelerated cardiovascular disease. The American public should not ignore the recommendations of the American Heart Association and of the various governmental agency reports that recommend a prudent diet low in cholesterol and saturated fat. And in this regard, I must disagree with the conclusions of the Food and Nutrition Board.

#### REFERENCES


1. National Academy of Sciences Food and Nutrition Board: Toward Healthful Diets. Washington, DC, Food and Nutrition Board, National Research Council, 1980

2. Connor WE, Connor SL: The key role of nutritional factors in the prevention of coronary heart disease. Prev Med 1:49-83, 1972

3. Keys A: Coronary heart disease—The global picture. Atherosclerosis 22:149-192, 1975

4. Stamler J: Population studies, *In* Levy RI, Riskind BM, Dennis BH, et al (Eds): Nutrition, Lipids and Coronary Heart Disease. New York, Raven Press, 1979, pp 25-28

5. Keys A: Coronary heart disease, serum cholesterol, and the diet. Acta Med Scand 207:153-160, 1980

6. Mahley RW: Cholesterol-induced hyperlipoproteinemia and atherosclerosis in dogs, swine and monkeys: Models for human atherosclerosis, *In* Gotto AM Jr, Smith LC, Allen B (Eds): Atherosclerosis—V. New York, Springer-Verlag, 1980, pp 355-358

7. Armstrong ML, Megan MB, Warner ED: Intimal thickening in normocholesterolemic rhesus monkeys fed low supplements of dietary cholesterol. Circ Res 34:447-454, 1974

8. Mahley RW: Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man, *In* Dietschy JM, Gotto AM Jr, Ontko JA (Eds): Disturbances in Lipid and Lipoprotein Metabolism. Bethesda, MD, American Physiological Society, 1978, pp 181-197

9. Mahley RW, Weisgraber KH, Innerarity T, et al: Characterization of the plasma lipoproteins and apoproteins of the erythrocebus patas monkey. Biochemistry 15:1928-1933, 1976

10. Mahley RW, Innerarity TL, Weisgraber KH: Alterations in metabolic activity of plasma lipoproteins. Ann NY Acad Sci 348: 265-277, 1980

11. Sherrill BC, Innerarity TL, Mahley RW: Rapid hepatic clearance of the canine lipoprotein containing only the E apoprotein by a high affinity receptor. J Biol Chem 255:1804-1807, 1980

12. Goldstein JL, Brown MS: The low-density lipoprotein pathway and its relation to atherosclerosis. Ann Rev Biochem 46:897-930, 1977

13. Mahley RW: Cholesterol feeding: Effects on lipoprotein structure and metabolism, *In* Gotto AM Jr, Smith LC, Allen B (Eds): Atherosclerosis—V. New York, Springer-Verlag, 1980, pp 355-358

14. Innerarity TL, Mahley RW: Enhanced binding by cultured human fibroblasts of apo-E containing lipoproteins as compared to low-density lipoproteins. Biochemistry 17:1440-1447, 1978

15. Weisgraber KH, Mahley RW: Subfractionation of human high density lipoproteins by heparin-Sepharose affinity chromatography. J Lipid Res 21:316-325, 1980

16. Mahley RW, Weisgraber KH: Subfractionation of high-density lipoprotein into two metabolically distinct subclasses by heparin-Sepharose affinity chromatography and Geon-Pevikon electrophoresis, *In* Lippel K (Ed): Report of the High Density Lipoprotein Methodology Workshop, NIH Publication, No. 79-1661. Washington, DC, US Dept of Health, Education, and Welfare, 1979, pp 356-366

17. Mahley RW, Innerarity TL, Bersot TP, et al: Alterations in human high-density lipoproteins, with or without increased plasma-cholesterol, induced by diets high in cholesterol. Lancet 2:807-809, 1978

18. Mahley RW, Nelson AW, Ferrans VJ, et al: Thrombosis in association with atherosclerosis induced by dietary perturbations in dogs. Science 192:1139-1141, 1976

19. Mahley RW, Innerarity TL, Weisgraber KH, et al: Canine hyperlipoproteinemia and atherosclerosis—Accumulation of lipid by aortic medial cells in vivo and in vitro. Am J Path 87:205-225, 1977

20. Mahley RW, Weisgraber KH, Innerarity T: Canine lipoproteins and atherosclerosis—II. Characterization of the plasma lipoproteins associated with atherogenic and nonatherogenic hyperlipidemia. Circ Res 35:722-733, 1974